SDS-Polyacrylamide Gel Electrophoresis （SDS-PAGE）

P38

Determining the MW of Protein by SDS-PAGE

Objective

Aim :

Comprehend the principle and application of Sodium dodecyl sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

- Preparation of PAG needs a long time, so do it at first.

Procedure

1. Reagent preparation:

2. Gel Cassette Assembly

(1) Clean and dry the glass plate, shelf etc.

(2) Assemble the gel apparatus

3. Preparation of the Gel and pour it into the sandwich layer of glass plate

3. Preparation of the Gel

(1) Prepare the Separating Gel (10%) 10 ml per gel

ddH_2O	3.90 ml
30% **Acrylamide stock solution**	3.33 ml
1.5M **Tris-Cl buffer (pH 8.8)**	2.50 ml
10% SDS	100 µl
10% AP	100 µl
10%TEMED	100 µl

Mix and pour the gel using a pipet,
allow to polymerize by overlaying gently with water.

Classification of PAGE

- Native PAGE
- Discontinuous PAGE
- SDS-PAGE
- IPG-IEF (Immobilized pH Gradient-IEF)
- 2-Dimensional Electrophoresis

Principle of PAGE

1. The basis of electrophoresis (vertical slab gels):

   A charged protein will migrate toward the oppositely charged region in an electric field.

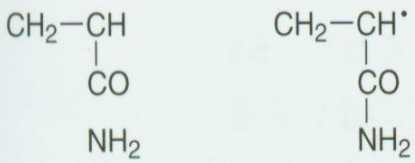
2. PAGE:

   Polyacrylamide: Acrylamide which forms a linear polymer, can be cross-linked with N,N`-methylene bisacrylamide, to form a gel matrix of controlled pore size. Polymerization is catalyzed by free radicals, generated by ammonium persulfate (AP) in the presence of TEMED.

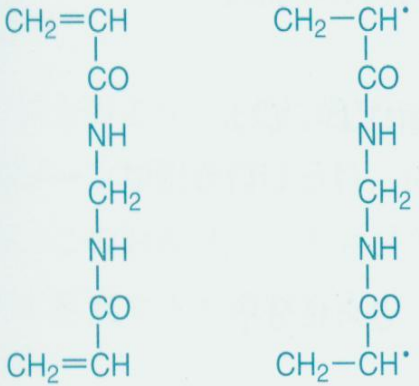
3. Acrylamide stock solutions with varying ratios of acrylamide and bis-acrylamide are routinely used, to create different pore size.

Formation of Polyacrylamide Gel

Acrylamide

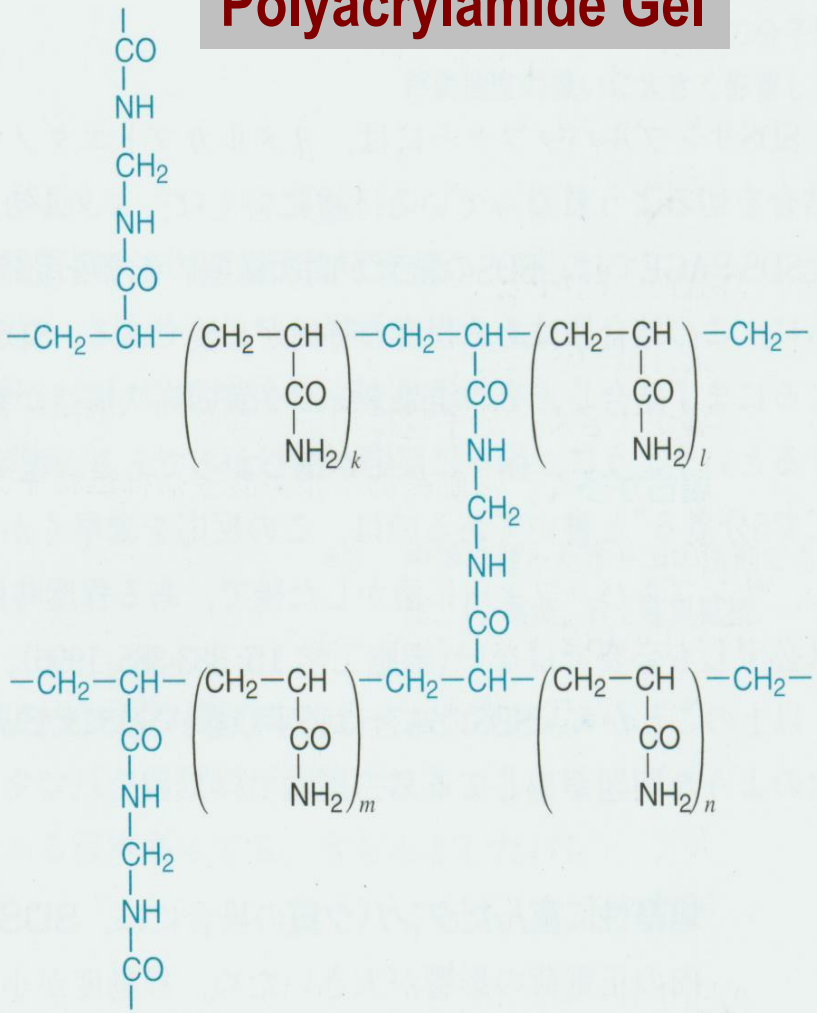


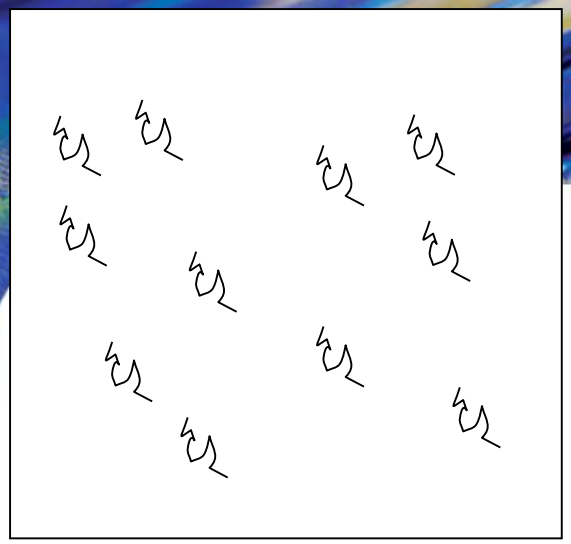
N,N'- methylene bis-acrylamide



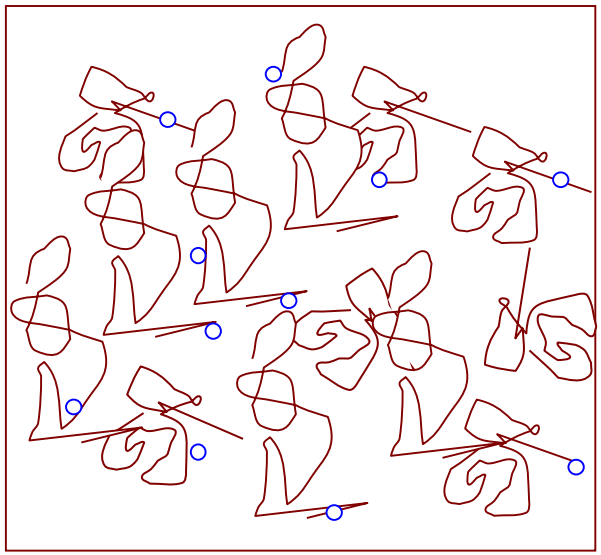
Polymerization

Polyacrylamide Gel

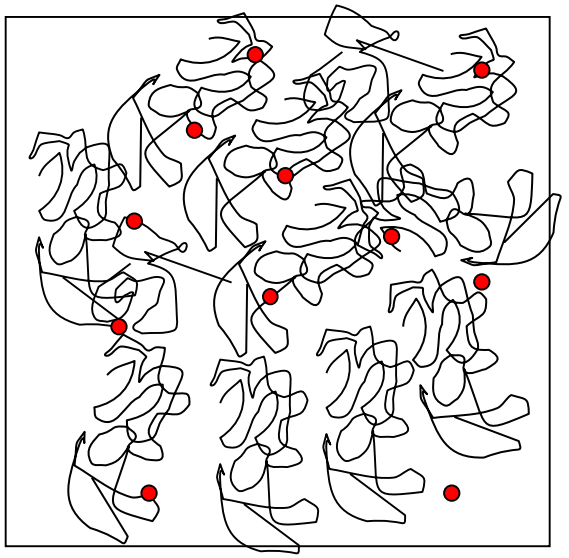




diluted solution



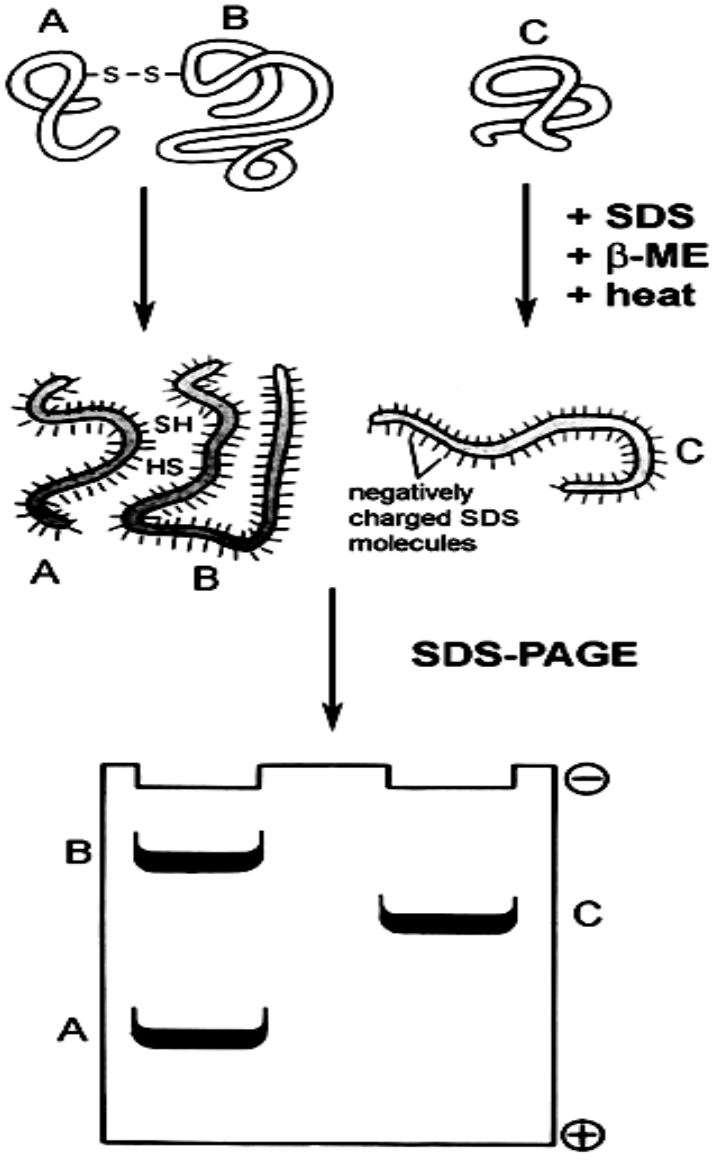
concentration —○— linker

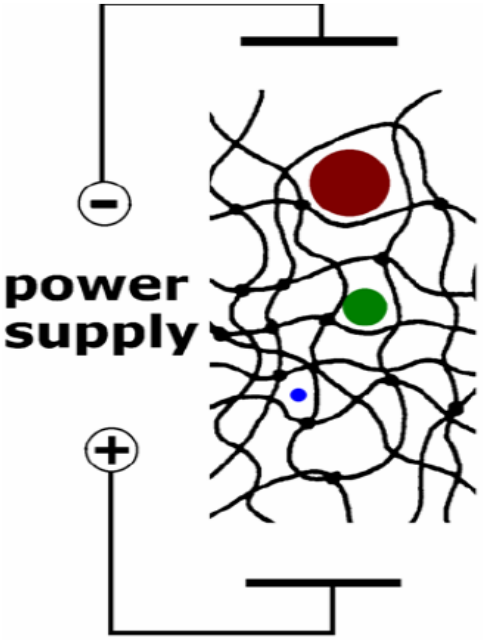


gel —●— joint

The Structure of Polyacrylamide Gel

Principle of PAGE

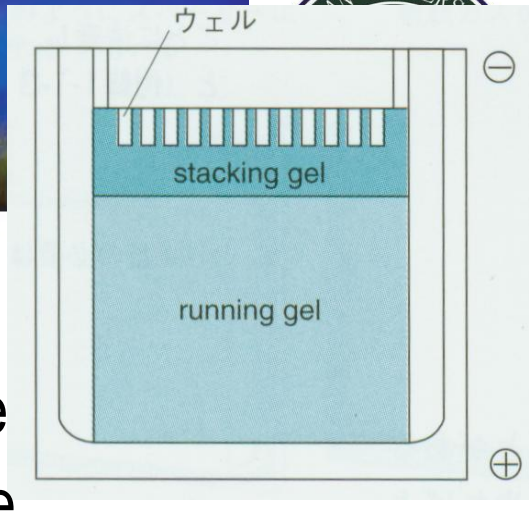




vertical slab gels

Principle of SDS-PAGE

4. SDS-PAGE: the exact rate of movement of a particular protein depends on its size.
5. SDS coats proteins with an approximately uniform charge-to-mass ratio of (-) charge , and proteins are approximately uniformly shaped into spheres, thus, proteins separated by SDS-PAGE are denatured.
6. Disulfides in proteins are broken by addition of ß-mercaptoethanol or 1, 4-dithiothreitol (DTT), so running proteins in SDS-PAGE are reduced condition.



7. A discontinuous gel system :

The Stacking Gel: approx. 10% of the volume of the total gel and a lower %(2.5-4.5%) acrylamide and a lower pH (6.8). charged molecules move freely and proteins in a sample should **accumulate** in stacks of closely spaced bands before encountering the separating gel.

The Separating gel: containing a higher % (7-15%) acrylamide and at a higher pH(8.8), proteins separate into discrete bands based on size.

The molecular weights of protein can be estimated by measuring the mobility of protein standards on the same gel.

Buffer System

- **Buffer for Stacking Gel:** 0.5 mol/L Tris-Cl, pH6.8
- **Buffer for Separating Gel:** 1.5 mol/L Tris-Cl, pH8.8
- **PAGE running buffer**（pH8.3）：

  0.025 mol/L Tris-Cl

  0.2 mol/L Glycine

2X Loading buffer

100 mM Tris-Cl (pH6.8)
200 mM DTT
4% SDS
0.2% Bromophenol blue
20% glycerol
(DTT should be added just before the buffer is used, from 1M stocking solution)

PAGE Procedure

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10%TEMED	100 µl

Mix and pour the gel using a pipet,
allow to polymerize by overlaying gently with water.

3. Preparation of the Gel

(2) Prepare the Stacking Gel 5.0% 5 ml per gel

ddH_2O	3.4 ml
30% **Acrylamide stock solution**	0.83 ml
1.5M **Tris-Cl buffer (pH 6.8)**	0.63 ml
10% SDS	50 μl
10% AP	50 μl
10%TEMED	50 μl

decant the water overlayer, pour the stacking gel, Insert the comb and allow to polymerize.

PAGE Procedure

(3) Once the gel has polymerized, the comb can be gently removed.

4. Sample preparation

(1) Combine 20 μl of sample (a unknown protein and a mix protein) with 20μl of protein loading buffer

(2) Heat samples for 3-5 min in a boiling water bath to denature the proteins, then cold and load the samples.

PAGE Procedure

5. Running the gel

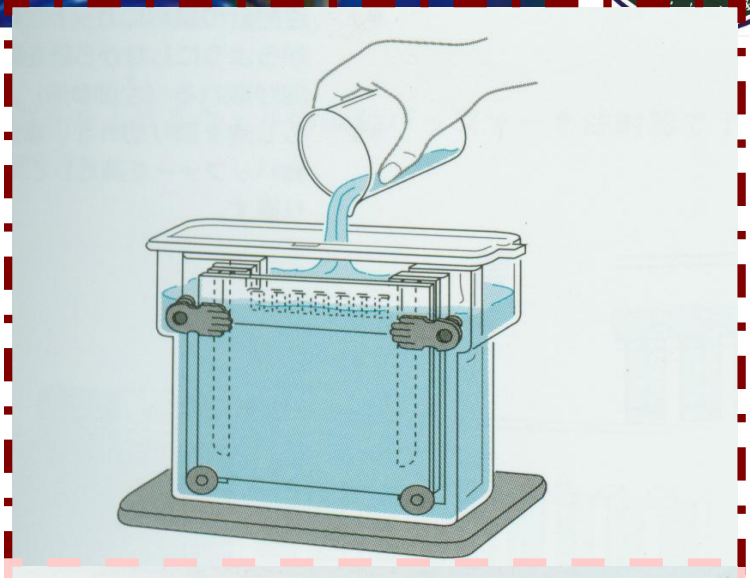
   (80V 0.5h; 150V 1.5V)

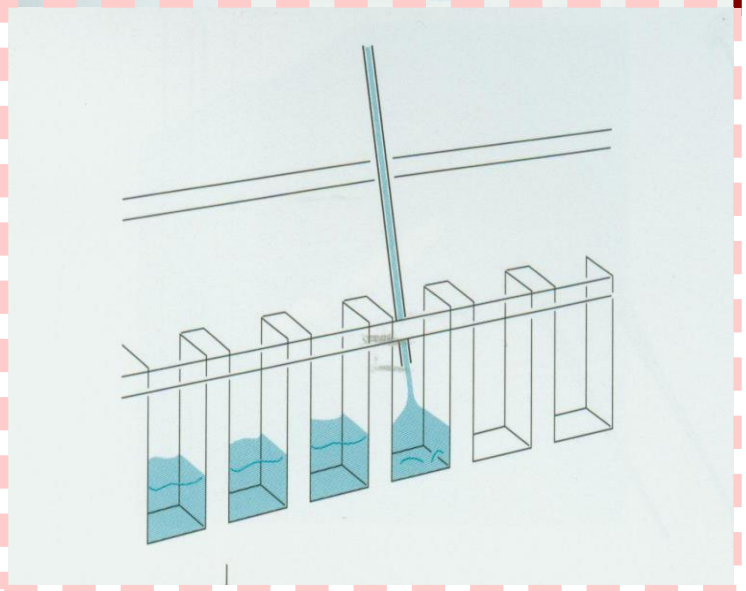
6. Coomassie Brilliant Blue Staining

   (Staining and destaining)

7. Observation

   (next week)





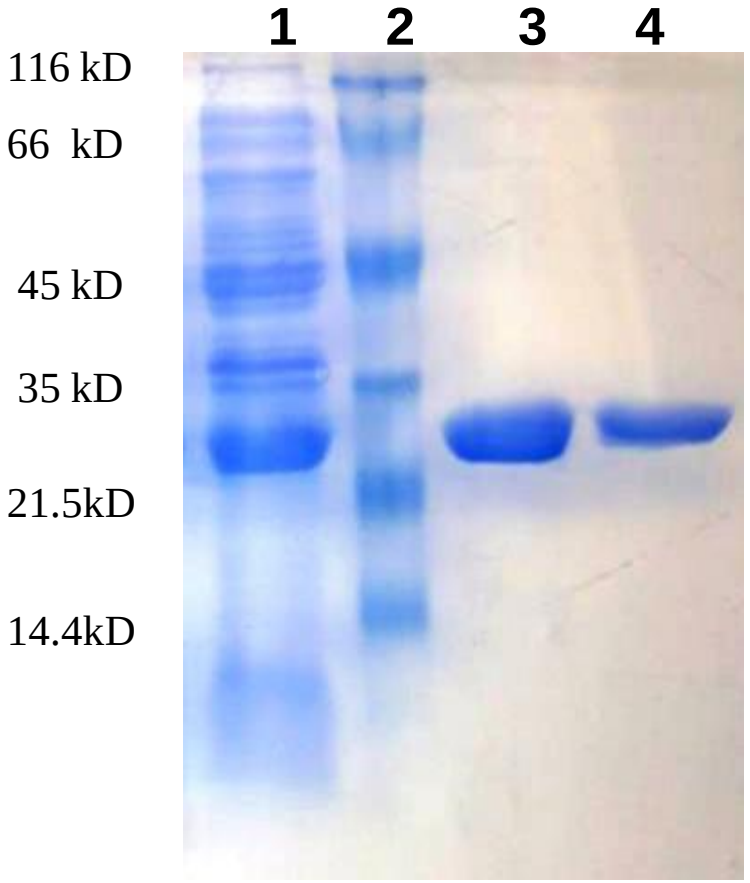
PAGE Procedure

During running the PAGE,

Please see a video of SDS-PAGE.

Result of SDS-PAGE

For example：



12% Separating Gel

Discussion

- If given a sample containing two proteins with large different mass weight, please list the methods for separating two proteins, and briefly introduce the principles.